Lead absorption and renal dysfunction in a South African battery factory

Rodney Ehrlich, Thomas Robins, Esme' Jordaan, Shirley Miller, Simphiwe Mbuli, Paul Selby, Sinclair Wynchank, Anthony Cantrell, Marc De Broe, Patrick D'Haese, Andrew Todd, Philip Landrigan

Department of Community Health, University of Cape Town, South Africa
R Ehrlich

Department of Environmental and Industrial Health, University of Michigan School of Public Health, USA
T Robins

South African Medical Research Council, Cape Town, South Africa
E Jordaan
P Selby
S Wynchank

Chemical Workers' Industrial Union, Johannesburg, South Africa
S Miller

Industrial Health Research Group, Cape Town, South Africa
S Mbuli

National Centre for Occupational Health, Johannesburg, South Africa
A Cantrell

Department of Nephrology-Hypertension, University of Antwerp, Belgium
M De Broe
P D'Haese

Department of Community Medicine, Mount Sinai School of Medicine, New York, USA
A Todd
P Landrigan

Corresponding author:
Dr RI Ehrlich, Department of Community Health, University of Cape Town Medical School, Observatory 7925, Cape Town, South Africa. Tel: 0027 21 406 6435; fax: 0027 21 406 6163; email: EHRLICH@ANAT.UCT.AC.ZA

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Abstract

***Objectives*—To test the association between inorganic lead (Pb) exposure, blood pressure, and renal function in South African battery factory workers, with both conventional and newer measures of renal function and integrity.**

***Methods*—Renal function measures included serum creatinine, urea, and urate (n = 382). Urinary markers (n = 199) included urinary N-acetyl-β-D-glucosaminidase (NAG), retinol binding protein, intestinal alkaline phosphatase, tissue non-specific alkaline phosphatase, Tamm-Horsfall glycoprotein, epidermal growth factor, and microalbuminuria.**

***Results*—Mean current blood Pb was 53.5 μg/dl (range 23 to 110), median zinc protoporphyrin 10.9 μg/g haemoglobin (range 1.9 to 104), and mean exposure duration 11.6 years (range 0.5 to 44.5). Mean historical blood Pb, available on 246 workers, was 57.3 μg/dl (range 14 to 96.3). After adjustment for age, weight and height, positive exposure response relations were found between current blood Pb, historical blood Pb, zinc protoporphyrin (ZPP), and serum creatinine and urate. Blood pressure was not associated with Pb exposure. Among the urinary markers, only NAG showed a positive association with current and historical blood Pb.**

***Conclusion*—An exposure-response relation between Pb and renal dysfunction across the range from <40 to >70 μg/dl blood Pb was found in this workforce, with conventional measures of short and long term Pb exposure and of renal function. This could not be explained by an effect on blood pressure, which was not associated with Pb exposure. The findings probably reflect a higher cumulative renal burden of Pb absorption in this workforce in comparison with those in recent negative studies. The results also confirm the need for strategies to reduce Pb exposure among industrial workers in South Africa.**
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With the control of acute inorganic lead (Pb) toxicity in many workforces exposed to Pb over the past few decades, attention has shifted to chronic or subclinical disorders attributable to Pb exposure.[1 2] Among these chronic effects, hypertension and renal dysfunction may be of particular concern in South African workers, because of the high prevalence of hypertension noted in some local population studies.[3 4]

Historically, chronic renal and hypertensive disease has been documented in people or populations with high Pb absorption.[1 5] Epidemiological studies have mostly not found an adverse effect of Pb on the kidney in exposed workers with concentrations of blood Pb of the order of <50–60 μg/dl,[1 6–13] which is the range into which the medical standards of several developed countries fall. One difficulty in showing such renal effects may be the insensitivity of traditional clinical tests of renal function in the detection of early kidney injury or altered function.[2] There has thus been considerable interest in potential laboratory biomarkers of early or site specific effects on the kidney of Pb absorption.[14 15]

The association between Pb and blood pressure, with or without renal dysfunction, has in turn been the subject of inconclusive epidemiological investigations, particularly in occupationally exposed groups with moderate levels of absorption.[1 16 17]

The objective of this study was to examine the association between (*a*) a variety of measures of Pb exposure or absorption and (*b*) blood pressure and renal integrity and function, in a South African workforce with a wide range of Pb exposure. As well as performing conventional renal function tests, a variety of urinary biomarkers of known or theoretical value in the measurement of early renal dysfunction or nephrotoxicity were measured.

Methods

A cross sectional study was conducted of 382 production employees of a Pb acid battery plant in the city of East London on the east coast of South Africa. The workforce was predominantly black,* most employees had grown up in rural districts of the Eastern Cape Province, and all were men. All permanent production workers were invited to participate, as well as temporary workers (mainly on short contracts) over 37 years of age. The age restriction was applied for statistical efficiency, by adding a group of older short service workers to the study group so as to reduce confounding by age. Although all of the subjects had some exposure to Pb, the wide range of exposure was

*Under apartheid, South Africans were divided into one of four racial groups; asian, black, coloured, and white. These catagories denote, to a varying extent, differences in socioeconomic status, diet, urbanisation. and experience of racial discrimination.

such as to permit examination of exposure-response associations.

The batteries are assembled by a conventional process. Exposures include inorganic Pb fume and Pb oxide, and 25%–35% sulphuric acid by weight in water. Measurements of Pb in air had been carried out in this plant only in recent years. A hygiene survey carried out six months before the study found measurements of Pb in air exceeding the South African occupational exposure limit of 0.15 mg/m^3 in 14 out of 30 samples. The median Pb in air concentration was 0.145 mg/m^3 and the range 0.01 to 5.48 mg/m^3.

QUESTIONNAIRE

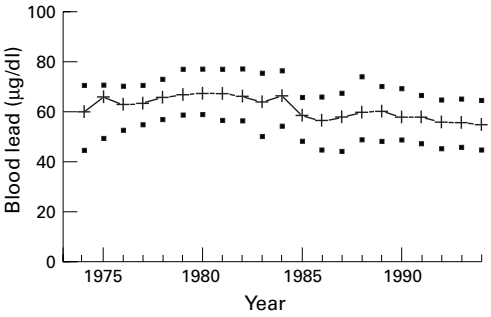
A questionnaire was administered by a single trained interviewer in the language of the subject (Xhosa, English, or Afrikaans). Questions covered demographic details, including periods lived in rural and urban areas; past medical illness including hypertension, diabetes mellitus, and kidney disease; analgesic use; smoking; alcohol use; physical activity; and dietary salt preference.

MEASUREMENT OF EXPOSURE AND ABSORPTION

Lead absorption measures included current blood Pb and zinc protoporphyrin (ZPP) concentrations, which can be regarded as reflecting a balance between recent and cumulative Pb absorption. Long term measures of exposure and absorption were based on the duration of work with Pb and the average of all past and current blood Pb results.

A detailed occupational history was taken of jobs in this plant and elsewhere, including questions about exposure to heavy metals both at and outside of work. Service periods were checked against company records, and discrepancies were remedied by recontacting the company and employee if necessary.

In the current study, heparinised whole blood was collected in Pb free Vacutainer tubes. Blood Pb was measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 5000) in the national quality control scheme reference laboratory, which in turn participates in the United Kingdom national external quality assessment scheme for Pb and cadmium (Cd). Zinc protoporphyrin was measured in the same specimen on a haematofluorometer (Aviv) and expressed in µg/g haemoglobin. Historical blood Pb measurements were available from 1974 to 1980 on fewer than five workers annually. Only from 1981 did the number of available blood results for any given year rise steadily, from 15 in 1981 to 377 in 1993. The median number of measurements per worker per year also rose from one to three. Most of these historical blood measurements had been done in one private laboratory with graphite furnace atomic absorption spectrophotometry. This laboratory participated in a national quality control scheme from 1979 onwards. Figure 1 shows the median blood Pb concentrations, with interquartile ranges. Although based on small numbers of specimens in the early years, the medians show a stable decline from the range



Median blood Pb concentrations, with interquartile ranges, 1974–94.

60 to 65 µg/dl between 1974 and 1984 to the range 55 to 59 µg/dl between 1985 and 1994.

Reasonably complete historical blood Pb information—that is, from the first year of employment, was available on a subset of subjects only (n=246). To avoid extrapolation to earlier periods, only this group was used to construct the historical blood Pb variables. These variables were (*a*) cumulative blood Pb (µg.y/dl), the sum of average blood Pb in each year over all such years of employment, and (*b*) historical blood Pb, calculated by dividing the cumulative blood Pb by duration of exposure.

BIOCHEMICAL AND HAEMATOLOGICAL TESTS

Serum urea, creatinine, uric acid, haemoglobin, and packed cell volume were measured by routine automated methods (appendix 1). Urine was screened qualitatively for protein, blood, and glucose with a test strip (Combur 10 test).

Urine was collected from a stratified random sample of 199 members of the study group. Stratification was by tertile of current blood Pb, so as to obtain a balanced distribution across the whole range of blood Pb. Urine was collected in plastic containers, buffered, and stored at 4°C for transport to the laboratory. Seven urinary biomarkers were measured quantitatively (appendix 1). Six of these are regarded as markers of tubular injury or dysfunction: N-acetyl-β-D-glucosaminidase (NAG), retinol binding protein, tissue non-specific alkaline phosphatase, intestinal alkaline phosphatase, Tamm-Horsfall glycoprotein, and epidermal growth factor. Microalbuminuria, a measure of glomerular dysfunction, was also measured. To standardise for varying diuresis, these biomarkers were all expressed /g urinary creatinine.

BLOOD PRESSURE, WEIGHT, AND HEIGHT

A calibrated mercury sphygmomanometer was used to measure blood pressure. A registered nurse was trained by one of the researchers, with the aid of a double headed stethoscope, to record systolic pressure at the beginning of the consecutive run of Korotkow sounds (phase I) and diastolic pressure as the disappearance of the sounds (phase V). Three recordings were made, the subject resting for five minutes before the first recording, and one minute between each. The average of the three readings was used for systolic and diastolic

Table 1 Exposure and lifestyle variables in a South African battery making workforce (n=382)

	Mean (SD)	*Median*	*Range*
Age (y)	41.2 (8.3)	41.0	21–71
Weight (kg)	75.5 (14)	73.2	47–148
Height (cm)	168.9 (6.3)	169.0	154–193
Lead exposure:			
Blood lead (μg/dl)	53.5 (12.7)	53.0	23–110
ZPP (μg/g Hb)	12.6 (9.3)	10.9	1.9–104
Duration of exposure (y)	11.6 (6.8)	10.5	0.5–44.5
Historical blood lead (μg/dl)*	57.3 (15)	58.7	14–96.3
Cumulative blood lead (μg.y/dl)*	579.0 (423)	518	7–2681
Smoking:			
Ever smoked (%)	57.6	—	—
Current (%)	52.4	—	—
Duration (y)	20.4 (8.7)	20	3–49
Pack-y	10.5 (7.5)	9	0.6–58
Alcohol:			
Ever alcohol (%)	70.4	—	—
Current (%)	67.0	—	—
Duration (y)	11.8 (11.0)	11.0	0–47
Weekly (g)	218 (237)	159.0	0–1566

*n=246. Hb=Haemoglobin.

blood pressure in the analysis. Weight and height were measured without shoes or shirt, on an industrial balance scale fitted with a vertical ruler.

TWO YEAR FOLLOW UP

About two years after the main study, a random sample of 40 workers from the original study group had bone Pb concentration measured in the left tibial midshaft with the technique of K *x* ray fluorescence (K-XRF).[18]

As a result of recent contention over the contribution of Cd to increased NAG activity in workers exposed to Pb,[19–21] a sample of 56 workers from the original study group had their blood Cd concentrations measured at two years. These 56 workers included 36 of the 40 subjects chosen for bone Pb testing, six others were followed up for persistent renal dysfunction, and 14 workers underwent annual medical surveillance. Cadmium was measured with graphite furnace atomic absorption spectrophotometry (Varian SpectrAA.30) in the same laboratory as was blood Pb in the main study.

STATISTICAL ANALYSIS

Spearman rank correlation coefficients (r) were used to examine correlation between exposure measures. Blood pressure was analysed in two ways. With blood pressure as a continuous variable, the analysis was conducted both with and without subjects on reported antihypertensive treatment. For use as a categorical variable and to ensure enough numbers for analysis, a liberal definition of hypertension was used—that is, subjects on reported antihypertensive treatment, or who had both a systolic pressure >140 mm Hg and a diastolic pressure >90 mm Hg.

Separate regression analyses were conducted for the association between systolic blood pressure, diastolic blood pressure, creatinine, urea, uric acid, the urinary biomarkers, haemoglobin, and packed cell volume, and each of the Pb exposure variables, controlling for potential confounders. The associations of continuous outcomes with exposure variables and covariates of interest were tested in multiple linear regression, with the maximum R^2 improvement technique to find the best subset of predictors. Logistic regression was used for hypertension as a categorical variable, as well as for the proportion of selected renal markers falling above upper reference levels. Age, height, and weight were entered into all of the models. Candidate covariates for the blood pressure regression included the alcohol and smoking measures, haemoglobin, physical exercise, dietary salt preference, years spent in an urban area, creatinine, urea, and uric acid. Candidate covariates entered into the renal outcome models included alcohol and smoking variables and diabetes.

Based on initial hypotheses, first order interactions between each of the Pb exposure variables and alcohol, smoking, and racial group were considered as candidate variables for entry into the relevant linear regression model.

ETHICS

The study was approved by the Ethics and Research Committee of the University of Cape Town. Signed informed consent was obtained from each participant. Subjects were individually informed of their results, and those with findings requiring action were counselled or referred to a medical practitioner.

Results

A total of 382 workers participated, of whom 359 were permanent and 23 temporary employees. Of the original invited group of 368 weekly paid (unionised) employees, six did not participate. Participation of monthly paid (supervisory) staff was not solicited; however, 20 out of 82 requested to take part. Of the subjects studied, 341 were black, the remainder being coloured or white. Historical blood Pb results from the first year of employment were available on 246 subjects.

Table 1 presents the age and Pb exposure status of the participants. The mean duration of service was 11.6 years, with 65% of the workforce having had between five and 15 years of exposure, and 12% fewer than five years of exposure. Exposure to Pb in jobs or activities outside of this company was uncommon.

Table 1 includes smoking and alcohol information. About half the workforce currently smoked, with a mean smoking history of 10.5 pack-years among smokers. A total of 67% of the workforce currently used alcohol, having an estimated mean weekly intake of 218 g alcohol among current users. (rough equivalents: 340 ml can of beer = 13 g alcohol; 175 ml glass of wine = 15 g; 50 ml glass of spirits = 17 g).

Table 2 presents renal function, haematological findings, and blood pressure. No reference range is provided for creatinine, as the upper limit of reference ranges for creatinine varies (somewhat arbitrarily) between 106 and 130 μmol/l in South African laboratories. The median serum creatinine in this group was 97 μmol/l, with 6.5% of results falling between 120 and 129 μmol/l and a further 3.7% were ⩾130 μmol/l. The proportion of subjects classified as hypertensive (blood pressure >140/90 mm Hg or on antihypertensive treatment) was 7.9%. Diabetes (defined as reporting diabetes or testing at least 1+ positive

*Table 2 Laboratory and clinical variables in a South African battery making workforce (n=382)**

	Mean (SD)	Median	Range	Laboratory range
Serum creatinine (μmol/l)	99.6 (16.2)	97.0	59–184	†
Serum urea (mmol/l)	5.6 (1.5)	5.5	2.4–17	2.5–6.7
Serum urate (μmol/l)	343.0 (329.0)	329.0	174–812	210–430
Haemoglobin (g/dl)	14.5 (1.2)	14.5	10.5–21.8	13.0–18.0
Packed cell volume (%)	42.7 (3.5)	42.6	31.6–62.9	40.0–54.0
Systolic blood pressure (mm Hg)	121.3 (13.6)	120.0	90–167	—
Diastolic blood pressure (mm Hg)	75.7 (10.0)	75.3	33–104	—

*See appendix 2 for conversion factors.
†See text.

*Table 3 Urinary biomakers in a South African battery making workforce (n=199)**

	Mean (SD)	Median	Range	Normal range	% Outside normal range
NAG (U/g creatinine)	4.0 (5.1)	2.9	0.7–62.9	<5.0	23.0
RBP (μg/g creatinine)	84.4 (122.9)	58.1	9.9–1291.5	<300	3.5
EGF (μg/g creatinine)	20.3 (11.1)	17.5	3.7–67.2	>10	11.0
THF (mg/g creatinine)	30.2 (25.4)	27	1.8–219.6	>10	14.0
IAP (U/g creatinine)	0.32 (0.48)	0.16	0–3.6	<2.0	2.0
TNAP (U/g creatinine)	0.1 (0.15)	0.07	0–1.6	<2.5	0
m-Alb (mg/g creatinine)	9.3 (19.3)	3.1	0.32–520.4	<25	5.0

*See text and appendix 1 for description. NAG=Nacetyl-β-D-glucosaminidase; RBP=retinol binding protein; EGF=epidermal growth factor; THF='Tamm-Horsfall glycoprotein; IAP=intestinal alkaline phosphatase; TNAP=tissue non-specific alkaline phosphatase; m-Alb=microalbuminuria.

for glycosuria with the dipstix method) was found in 2.9% of the group.

Table 3 presents the urinary markers, with the normal reference concentrations supplied by the laboratory. Of note is that 23% of the NAG test results fell above the normal range.

The different dimensions of Pb exposure represented by the Pb variables are reflected in their intercorrelations. Current blood Pb was moderately strongly correlated with ZPP (r=0.67) and historical blood Pb (r=0.66), less strongly with cumulative blood Pb (r=0.43), and weakly with duration (r=0.18). Cumulative blood Pb was highly correlated with duration (r=0.94), moderately strongly with historical blood Pb (r=0.58), and less strongly with ZPP (r=0.32). All correlations were significant ($p < 0.05$).

There was no significant association between any of the Pb exposure measures and either systolic or diastolic blood pressure, or hypertension defined categorically. After modelling, both systolic and diastolic blood pressure were positively associated in a multivariable regression model with age, weight, and weekly alcohol intake. Also, systolic blood pressure was positively associated with serum uric acid. There was no association between blood pressure and haemoglobin, smoking, physical activity, dietary salt preference, or years spent in an urban area.

Table 4 shows the associations between the Pb exposure variables and renal outcome variables from multiple linear regression analysis. For clearer exposition of exposure-response relations, the Pb exposure variables are categorised, and the renal outcomes adjusted for age, height, and weight.

Current blood Pb, ZPP, and historical blood Pb were all positively associated with both serum creatinine and uric acid. In the case of current and historical blood Pb, the exposure response association is apparent across the whole range from <40 μg/dl to >70 μg/dl. In the case of ZPP the range extends from <5 μg/g Hb to >25 μg/g Hb. Entering weekly alcohol intake into the regression models for uric acid reduced the coefficient for current blood Pb slightly (from 1.55 to 1.36) but had otherwise no confounding effect.

The only significant association between urea and any of the Pb exposure variables was with ZPP (partial r=0.2, p=0.0001). Neither cumulative blood Pb nor duration of exposure was associated with creatinine, urea, or uric acid.

Urinary markers were tested in 199 subjects. There was no significant difference between those tested and the rest of the workforce (n=183) for mean current blood Pb (53.1 μg/dl *v* 54.0 μg/dl) or duration (11.4 *v* 12.0 y). Of the urinary markers, only NAG showed a positive association with current blood Pb (r=0.18, p=0.01) and historical blood Pb (r=0.21, p=0.01), but not with the other Pb exposure variables. These trends were not significant once the associations were modelled (table 4). The association of NAG with the percentage change in blood Pb concentration between the second last and last blood Pb measurement was also examined, in view of a recent finding along these lines.[19] The median interval between the last two blood Pb values was 7.9 months and the median percentage change 3.5% (range -42 to 87). No association between NAG and recent change in blood Pb was found (r=-0.06, p=0.3). Epidermal growth factor was negatively associated with cumulative blood Pb (r= -0.18, p=0.04). No associations between Pb

*Table 4 Renal markers and lead exposure in a South African battery making workforce**

	n	Serum creatinine (μmol/l) Mean (SE)	Serum uric acid (μmol/l) Mean (SE)	n	Urinary NAG (U/g creatinine) Mean (SE)
Current blood lead (μg/dl):					
23–40	48	95.4 (2.2)	313.6 (12.1)	27	2.9 (0.5)
41–50	112	97.8 (1.5)	334.9 (7.9)	61	3.5 (0.3)
51–60	115	100.2 (1.4)	339.2 (7.8)	59	3.9 (0.3)
61–70	73	101.4 (1.8)	370.9 (9.8)	37	4.2 (0.5)
71–110	28	108.3 (3.0)	378.4 (15.7)	11	5.8 (0.9)
p For trend		0.008	0.0006		0.09
ZPP (μg/g Hb):					
1.9–4.9	54	95.1 (2.0)	329.1 (11.4)	30	3.3 (0.5)
5–9.9	110	96.7 (1.4)	338.8 (8.0)	51	4.0 (0.4)
10–14.9	90	98.7 (1.5)	338.9 (8.8)	49	3.3 (0.4)
15–19.9	47	100.3 (2.1)	343.2 (12.2)	26	4.4 (0.6)
20–24.9	33	104.2 (2.6)	343.6 (14.6)	16	3.5 (0.7)
25–104	30	115.3 (2.7)	396.3 (15.3)	17	4.8 (0.7)
p For trend		0.02	0.006		0.4
Duration (y):					
0.5–1.9	26	96.3 (3.2)	327.9 (17.0)	19	3.1 (0.7)
2–4.9	20	98.5 (3.7)	358.2 (19.8)	10	2.2 (0.9)
5–9.9	131	99.2 (1.4)	340.2 (7.8)	62	4.0 (0.4)
10–14.9	116	99.8 (1.5)	344.5 (7.9)	62	3.8 (0.3)
15–44.5	89	101.2 (1.8)	348.1 (9.7)	45	3.8 (0.4)
p For trend		0.7	0.7		0.3
Cumulative blood lead (μg.y/dl):					
7–352.3	64	97.6 (2.0)	332.1 (11.1)	32	3.1 (0.4)
352.4–533.3	65	97.5 (2.0)	329.0 (11.0)	33	3.5 (0.4)
533.4–729.8	62	101.9 (2.0)	342.3 (11.2)	36	3.8 (0.4)
729.9–2681	55	101.3 (2.3)	359.5 (12.7)	24	4.2 (0.5)
p For trend		0.3	0.3		0.4
Historical blood lead (μg/dl):					
14–40	32	94.6 (2.8)	309.1 (15.4)	19	3.0 (0.5)
41–50	35	98.3 (2.7)	361.9 (14.9)	18	2.7 (0.6)
51–60	67	96.4 (1.9)	324.5 (10.6)	33	3.5 (0.4)
61–70	57	101.4 (2.1)	338.8 (11.5)	33	3.9 (0.4)
71–96.3	55	105.0 (2.1)	364.0 (11.8)	22	4.6 (0.5)
p For trend		0.01	0.01		0.1

*Adjusted in linear regression for age, height, and weight.
NAG = N-acetyl-β-D-glucosaminidase; ZPP = zinc protoporphyrin; Hb = haemoglobin.

*Table 5 Prevalence of abnormal renal markers and lead exposure in a South African battery making workforce**

	Serum creatinine ≥125 μmol/l Prevalence			*Serum uric acid ≥500 μmol/l Prevalence*		*Urinary NAG ≥5 U/g creatinine Prevalence*		
	n	*(%)*	*OR (95% CI)*	*(%)*	*OR (95% CI)*	*n*	*(%)*	*OR (95% CI)*
Current blood lead (μg/dl):								
23–50	160	2.5	1.0	1.9	1.0	88	20.5	1.0
51–60	115	7.8	3.3 (0.99 to 11.1)	5.2	2.8 (0.6 to 11.8)	59	22.0	1.0 (0.4 to 2.3)
61–110	101	9.9	4.4 (1.3 to 14.5)	11.9	7.9 (2.1 to 29.4)	48	29.2	1.6 (0.7 to 3.6)
ZPP (μg/g Hb):								
1.9–10	166	1.8	1.0	2.4	1.0	82	20.7	1.0
10.1–20	135	5.2	2.8 (0.7 to 11.3)	4.4	1.7 (0.4 to 6.3)	74	24.3	1.1 (0.5 to 2.5)
20.1–104	64	20.3	13.5 (3.6 to 50.4)	15.6	7.6 (2.2 to 26.5)	33	27.3	1.3 (0.5 to 3.5)
Duration (y):								
0.5–7.5	124	4.9	1.0	2.4	1.0	64	21.9	1.0
7.6–12.5	112	3.6	0.6 (0.1 to 2.3)	3.6	1.3 (0.2 to 6.1)	57	28.1	1.4 (0.6 to 3.2)
12.6–44.5	146	8.9	1.3 (0.4 to 4.1)	9.6	3.2 (0.8 to 12.8)	77	19.5	0.7 (0.3 to 1.8)
Cumulative blood lead (μg.y/dl):								
7–520.0	123	4.1	1.0	4.1	1.0	63	19.1	1.0
520.1–2681	123	8.9	2.0 (0.6 to 6.3)	6.0	1.4 (0.4 to 4.5)	62	27.4	1.7 (0.7 to 4.1)
Historical blood lead (μg/dl):								
14–60	134	3.0	1.0	1.5	1.0	70	15.7	1.0
61–96.3	112	10.7	3.7 (1.1 to 12.0)	9.8	7.1 (1.5 to 33.8)	55	32.7	2.7 (1.1 to 6.6)

*OR derived from a multiple logistic regression model, adjusted for age, height, and weight.
NAG = N-acetyl-β-D-glucosaminidase; ZPP = zinc protoporphyrin; Hb = haemoglobin.

exposure and any of the other urinary markers were found.

The findings in table 4 were re-examined by categorising the renal outcomes in terms of the proportion that fell above a normal upper limit (table 5). The number of exposure categories had to be reduced to accomodate the relatively few abnormal observations. The findings with this analysis were consistent with the trends found in table 4.

In an analysis of haematological outcomes, ZPP was negatively associated with both packed cell volume and haemoglobin. Alcohol use and smoking were positively associated with packed cell volume, and alcohol use and weight with haemoglobin concentration.

EFFECT MODIFICATION

There was no interaction between alcohol and Pb in the effect on renal function or any of the urinary markers. Similarly, although smoking was associated with blood Pb (r=0.12, p<0.05, for current smoking and blood Pb), there was no interaction between any of the smoking variables and Pb in the associations with renal outcomes.

Examination of an interaction between racial group and Pb exposure in the association with renal outcomes was limited by the few employees who were not black (n=41). After excluding this group from the analysis, there was essentially no change in the associations found. The key analyses were repeated also after excluding the 20 monthly paid employees, with no change in the results.

MEASUREMENTS AT TWO YEARS

The mean (SD) tibial bone Pb in the random sample of 40 subjects measured at two years was 69.7 (35.5) μg/g bone mineral, and median (range) 60.9 μg/g (22.6 to 179.4). Of the 56 blood Cd concentrations measured, 38 fell below the laboratory's level of detection of 0.5 μg/l, a further 17 were between <0.5 μg/l and 0.9 μg/l, and one reached 1.2 μg/l.

Discussion

The main finding in this study is that blood Pb concentration, current and historical, and ZPP are associated with measures of renal impairment or injury, but not with blood pressure, in a South African workforce.

The threshold, if any, above which Pb absorption produces renal impairment is controversial. It has been suggested that maintenance of blood Pb concentrations <70 μg/dl is sufficient to protect against adverse renal effects in most male workers.[12] Thresholds are difficult to define, however, as no single measure of Pb exposure reflects the true cumulative dose to the kidney in all exposure situations.

In this workforce, with a mean duration of exposure of 11.6 years, and in which half the workforce had blood Pb measurements (measured currently or historically) of <60 μg/dl, robust exposure-response relations were found between serum creatinine and the Pb exposure measures assumed to have a substantial short term component—that is current blood Pb and ZPP. These associations were monotonic down to <40 μg/dl and 5 μg/g Hb respectively. Serum urea seemed to be influenced by ZPP, but not by blood Pb.

The incompleteness of historical blood Pb information on the longer service workers was dealt with by restricting the analysis to workers on whom information on long term or cumulative measures of Pb exposure was available. Assuming that blood Pb concentrations were higher in the years before routine blood Pb testing than in recent years, the resulting censored group are likely to have had cumulative blood Pb and historical blood Pb concentrations that were lower than those of the workforce as a whole. However, this restriction would have reduced the power of the study to detect an effect. Systematic and random error could also have been introduced by varying indications for routine testing and by variability in the quality of laboratory measurement over time.

Nevertheless, a similar exposure-response relation was found between historical blood Pb and creatinine as for the measures which included short term exposure components. However, renal function seemed to be uninfluenced by duration of exposure, and this was reflected also in the lack of association with cumulative blood Pb.

Serum creatinine is a function of muscle mass and diet, as well as the glomerular filtration rate.[22] Serum creatinine adjusted for age and weight, as was done in this study, provides a reasonable reflection of creatinine clearance.[23] The increase in mean creatinine (and therefore decline in mean clearance) with each increment in blood Pb of 10 μg/dl (or in ZPP of 5 μg/g) was small in this workforce. It is thought, however, that by the time serum creatinine rises above the clinical reference range, up to 60% of nephrons may be dysfunctional.[24] The cumulative effect on the kidney of aging, Pb exposure, and other renal insults may thus become clinically important in some people, justifying measures aimed at preventing subclinical effects in exposed workers.

Uric acid too was found to show exposure-response relations with current and historical blood Pb and ZPP, which if causal may reflect an influence of Pb on the renal handling of urate. This handling is complex and may include glomerular filtration, reabsorption, tubular secretion, and postsecretory reabsorption.[25] The mechanism of hyperuricaemia in Pb nephropathy is postulated to be due to increased tubular reabsorption of uric acid.[26] Hyperuricaemia associated with increased Pb absorption could thus be interpreted at least in part as a measure of tubular dysfunction.

By contrast with the renal findings, there was no association between Pb and blood pressure in this workforce. Although a substantial proportion of the workforce had grown up in rural areas, the age standardised hypertension prevalence of 6.0% with the World Health Organisation definition (blood pressure ⩾165/90) was only slightly lower than the 6.8% found in a comparable but more urbanised population in Cape Town,[27] suggesting little protective effect of a rural background. These hypertension prevalences are in turn low compared with those found in comparable population studies elsewhere in South Africa.[3 4]

We considered non-occupational sources of Pb among these subjects. Domestic sources of Pb in folk remedies and home brewed beer, or in batteries burned for fuel or in backyard smelting of Pb, are possible sources, but there was no evidence to confirm any of these. Other possible environmental or occupational nephrotoxic exposures include Cd[20] and arsine.[28] Arsenic contamination of Pb was very low in the materials used in this factory. Cadmium is discussed later. Also, although alcohol intake was high in the workforce, and alcohol was independently associated with blood Pb, uric acid, and blood pressure, no interaction of alcohol with Pb in the effect on renal function was found.

Another aim of the study was to determine if the use of urinary biomarkers was of any value in detecting and localising disturbance of the proximal tubules and other regions of the nephron by Pb. The NAG activity includes that of two isoenzymes, reflective of different modes of disruption of cellular processes in the proximal tubules.[19 20] Retinol binding protein is a low molecular weight protein derived from serum reflecting tubular, particularly proximal tubular, damage.[15] Other tubular markers are the isoforms of alkaline phosphatase, tissue non-specific alkaline phosphatase, and intestinal alkaline phosphatase, localised in the brush border of the proximal tubular cells. Tissue non-specific alkaline phosphatase is present mainly in the proximal convoluted tubule (S1-S2 segments), whereas intestinal alkaline phosphatase is found exclusively in the straight part of the proximal tubule (S3 segment).[14]

Of these proximal tubular markers, NAG activity in the urine was above the reference range in almost a quarter of the samples tested and showed some correlation with current and historical blood Pb, although not with recent changes in blood Pb as has been suggested.[19] Although an increased urinary NAG activity has been found to be associated with some measure of Pb exposure in several studies[8 11–13 19 29] only a few have shown an exposure-response correlation of NAG with blood Pb.[8 13] The interpretation of the change in NAG in workers exposed to Pb is thus contentious, and it has been argued that the apparent association is due to a confounding effect on the kidney of Cd intake.[11 12 19 21] In this study there was no Cd in the production process, and the median blood Cd concentration, a measure of recent absorption tested two years after the main study, was similar to concentrations in the non-smoking general population of 0.4-1.0 μg/l.[30] It seems implausible that Cd at such low concentrations could entirely explain the association, although limited, found between blood Pb and NAG in this population. However, as Cd concentrations were not measured at the same time as Pb in the main study, confounding could not be examined directly.

Tamm-Horsfall glycoprotein is found in the thick ascending limb of the loop of Henle and the early distal tubule,[31] whereas the polypeptide epidermal growth factor seems to be associated with the thick ascending limb and distal tubule as well as the collecting ducts.[32] Reduced expression of Tamm-Horsfall glycoprotein and epidermal growth factor is thought to reflect renal medullary dysfunction or early damage in these regions. Epidermal growth factor alone was negatively correlated with cumulative blood Pb, but not with any of the shorter term Pb exposure measures. Finally, microalbuminuria (albuminuria varying between 30 and 300 mg/24 hours) was measured as a reflection of glomerular damage; no association with Pb exposure was found.

Of the Pb exposure measures, blood Pb concentration (current or historical) and ZPP were consistent predictors of adverse renal effects in this study, with no absolute threshold for the appearance of such effects. The findings

regarding creatinine are similar in some respects to those of Lilis *et al*[6] among secondary Pb smelter workers (21% with blood Pb concentration >60 μg/dl, but heavy previous exposure) and Pinto de Almeida *et al*[33] among primary smelter workers in Brazil (mean blood Pb 62 μg/dl). In both of these studies, positive associations between creatinine and ZPP were shown, whereas the association of creatinine with blood Pb concentration was less consistent.

By contrast, in studies of various groups exposed to Pb in Europe and Singapore with varying durations of exposure, in which the mean blood Pb concentrations were lower than in this study (between 30 and 51 μg/dl), no association between serum creatinine and Pb exposure was found.[8–10 12 13 19]

The findings in this study are consistent with a subclinical renal response to Pb that may start at blood Pb concentrations of between 40 and 50 μg/dl. This was found for serum creatinine and urate, measures of relatively late disturbance of nephron function, as well as NAG, an early marker of proximal tubule disturbance. The most plausible explanation for the discrepancy between this study and the negative studies cited is the presence of a higher cumulative body burden of Pb in this workforce. It is probable that average past exposure in this workforce was heavy, as control regulation for occupational Pb was introduced into South African industry only in 1991,[34] setting the medical standard at 80 μg/dl blood Pb.

Body burden in the form of bone Pb relative to renal function has recently begun to be measured in cross sectional workforce studies. In one study, no association between tibial Pb and renal function was found,[10] whereas in the other tibial Pb was positively associated with a slight hyperfiltration state.[12] Tibial bone Pb measurements done on a random fraction of the workforce in this study two years after the main study showed a median bone Pb concentration of 60.9 μg/g. This is higher than has been reported in most occupational groups.[10 18 35] Surprisingly, it is similar to the tibial Pb concentrations reported among Belgian Pb smelter workers (geometric mean 65.8 μg/g, range 19.6 to 167.7).[12] However, blood Pb and ZPP concentrations in the Belgian workers were much lower than in this study, suggesting that both current Pb exposure and the bone Pb store may contribute to the toxicologically active pool of Pb. In a currently exposed workforce with heavy past exposure, blood Pb may thus remain a reasonable measure of renal Pb burden, whereas in less heavily exposed populations, either historically or currently, or in people no longer exposed, blood Pb is a poor measure of renal Pb burden.[36 37] Similarly, ZPP can be regarded as a measure integrating both recent and more remote Pb exposure and reflecting the biologically active renal Pb burden.[38]

The search for a coherent theory of Pb nephrotoxicity is somewhat complicated by recent population studies which have shown an association between creatinine[39 40] and creatinine clearance[39] and blood Pb at very low concentrations, of the order of 10 μg/dl, as well as with ZPP of the order of 1.0 μg/g Hb. It is speculated that if real, these associations may be indicative of a biphasic response of the kidney to Pb, one at higher concentrations found in occupational groups and one at the very low concentrations typical of the general population.[40]

Finally, this study confirms the need to amend the South African medical blood Pb standard of 80 μg/dl downwards towards internationally accepted levels.[34]

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Appendix 1: Laboratory methods

Serum biochemistry was measured on a Technicon RAXT autoanalyser by the following methods: creatinine, Jaffe rate method; urea, urease method linked to the oxidation of NADH; uric acid, uricase method with production of hydrogen peroxide reacting quantitatively to form a quinoimine dye. Haemoglobin was measured colorimetrically on a Technicon H1 autoanalyser.

N-acetyl-β-D-glucosaminidase was measured with a colorometric kit (Boehringer Mannheim, Germany). Retinol binding protein was measured by an automated continuous flow non-isotopic immunoassay based on latex particle agglutination. Tissue non-specific alkaline phosphatase and intestinal alkaline phosphatase were measured with two commercially available enzyme linked immunosorbent assay (ELISA) kits (Innogenetics, Belgium), Tamm-Horsfall glycoprotein, and microalbuminuria by ELISA (Elias, Germany), and epidermal growth factor by competitive ELISA. Urinary creatinine was measured by a modified Jaffe reaction. (Further details available from authors on request).

Appendix 2: SI conversion factors

Blood lead	1 μmol/l = 20.7 μg/dl
Zinc protoporphyrin	1 μmol/l = 62.6 μg/dl
Creatinine	88.4 μmol/l = 1 mg/dl
Urea	0.166 mmol/l = 1 mg/dl
Uric acid	60 μmol/l = 1 mg/dl

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